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SCHEMATIC REPRESENTATION OF THE PHAGEMID VECTOR pCES1

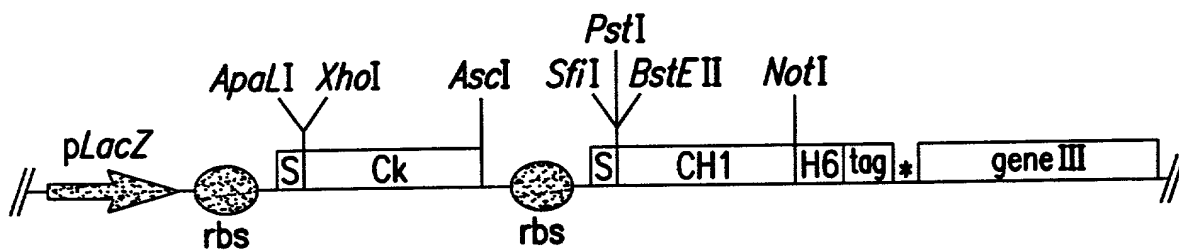


FIG. 1A

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POLYLINKER REGION OF THE PHAGEMID VECTOR pCES1

Signal sequence
 --- TTA TTC GCA ATT CCT TTA GTT GTT CCT TTC TAT TCT CAC AGT GCA CAG GTC CAA CTG CAG GTC GAC CTC GAG
 L F A I P L V V P F Y S H S A Q V Q L Q V D L E
 XhoI

ApalI +1
 ATC AAA CGT GGA ACT GTG --- GGA GAG TGT TAA TAA GGC GCG CCA ATT CTA TTT CAA GGA GAC AGT CAT A
 I K R G T V G E C * *
 Human Ck gene Stops

AscI
 ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA CTC GCG GCC CAG CCG GCC ATG GCC CAG GTG
 M K Y L L P T A A A G L L L L A A Q P A M A Q V
 SfiI +1

NotI
 CAG CTG CAG GAG AGC GGG GTC ACC GTC TCA AGC GCC TCC ACC --- AAA TCT TGT GCG GCC GCA CAT CAT CAT CAT
 Q L Q E S G V T V S S A S T K S C A A A H H H H
 Human CHI (γ1) gene Hexahistidine

BstEII
 CAT CAC GGG GCC GCA GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT GGG GCC GCA TAG ACT GTT ---
 H H G A A E Q K L I S E E D L N G A * T V
 Tag c-Myc Tag Amber GeneIII

FIG.1B

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Oligonucleotide primers used for construction of the library

A. Primary amplifications

IgM heavy chain constant region

HuIgMFOR 5'-TGG AAG AGG CAC GTT CTT TTC TTT-3'

κ light chain constant region

HuCKFOR 5'-ACA CTC TCC CCT GTT GAA GCT CTT-3'

λ light chain constant region

HuCI2-FOR 5'-TGA ACA TTC TGT AGG GGC CAC TG-3'

HuCI7-FOR 5'-AGA GCA TTC TGC AGG GGC CAC TG-3'

VH back

HuVH1B/7A-BACK 5'-CAG RTG CAG CTG GTG CAR TCT GG-3'

HuVH1C-BACK 5'-SAG GTC CAG CTG GTR CAG TCT GG-3'

HuVH2B-BACK 5'-CAG RTC ACC TTG AAG GAG TCT GG-3'

HuVH3B-BACK 5'-SAG GTG CAG CTG GTG GAG TCT GG-3'

HuVH3C-BACK 5'-GAG GTG CAG CTG GTG GAG WCY GG-3'

HuVH4B-BACK 5'-CAG GTG CAG CTA CAG CAG TGG GG-3'

HuVH4C-BACK 5'-CAG STG CAG CTG CAG GAG TCS GG-3'

HuVH5B-BACK 5'-GAR GTG CAG CTG GTG CAG TCT GG-3'

HuVH6A-BACK 5'-CAG GTA CAG CTG CAG CAG TCA GG-3'

B. Secondary amplifications

κ light chain constant region
 HuCK-FOR-ASC

λ light chain constant region
 HuCI2-FOR-ASC
 HuCI7-FOR-ASC

VH back

HuVH1B/7A-BACK-SFI

HuVH1C-BACK-SFI

HuVH2B-BACK-SFI

HuVH3B-BACK-SFI

HuVH3C-BACK-SFI

HuVH4B-BACK-SFI

HuVH4C-BACK-SFI

HuVH5B-BACK-SFI

HuVH6A-BACK-SFI

FIG.2i

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Oligonucleotide primers used for construction of the library

VH forward		
		HuJH1/2-FOR
		HuJH3-FOR
		HuJH4/5-FOR
		HuJH6-FOR
Vκ back		
		HuVk1B-BACK-APA
		HuVk2-BACK-APA
		HuVk3B-BACK-APA
		HuVk4B-BACK-APA
		HuVk5-BACK-APA
		HuVk6-BACK-APA
Vκ back		
HuVk1B-BACK	5'-GAC ATC CAG WTG ACC CAG TCT CC-3'	
HuVk2-BACK	5'-GAT GTT GTG ATG ACT CAG TCT CC-3'	
HuVk3B-BACK	5'-GAA ATT GTG WTG ACR CAG TCT CC-3'	
HuVk4B-BACK	5'-GAT ATT GTG ATG ACC CAC ACT CC-3'	
HuVk5-BACK	5'-GAA ACG ACA CTC ACG CAG TCT CC-3'	
HuVk6-BACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3'	

FIG.2ii

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Oligonucleotide primers used for construction of the library

V λ back		V λ back
HuV λ 1A-BACK	5'-CAG TCT GTG CTG ACT CAG CCA CC-3'	HuV λ 1A-BACK-APA
HuV λ 1B-BACK	5'-CAG TCT GTG YTG ACG CAG CCG CC-3'	HuV λ 1B-BACK-APA
HuV λ 1C-BACK	5'-CAG TCT GTC GTG ACG CAG CCG CC-3'	HuV λ 1C-BACK-APA
HuV λ 2-BACK	5'-CAR TCT GCC CTG ACT CAG CCT-3'	HuV λ 2-BACK-APA
HuV λ 3A-BACK	5'-TCC TAT GWG CTG ACT CAG CCA CC-3'	HuV λ 3A-BACK-APA
HuV λ 3B-BACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3'	HuV λ 3B-BACK-APA
HuV λ 4-BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3'	HuV λ 4-BACK-APA
HuV λ 5-BACK	5'-CAG GCT GTG CTG ACT CAG CCG TC-3'	HuV λ 5-BACK-APA
HuV λ 6-BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3'	HuV λ 6-BACK-APA
HuV λ 7/8-BACK	5'-CAG RCT GTG GTG ACY CAG GAG CC-3'	HuV λ 7/8-BACK-APA
HuV λ 9-BACK	5'-CWG CCT GTG CTG ACT CAG CCM CC-3'	HuV λ 9-BACK-APA

FIG.2iii

Oligonucleotide primers used for construction of the library

5'-ACC GCC TCC ACC GGG CGC GCC TTA TTA ACA CTC TCC CCT GTT GAA GCT CTT-3'

5'-ACC GCC TCC ACC GGG CGC GCC TTA TTA TGA ACA TTC TGT AGG GGC CAC TG-3'

5'-ACC GCC TCC ACC GGG CGC GCC TTA TTA AGA GCA TTC TGC AGG GGC CAC TG-3'

5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG RTG CAG CTG GTG CAR TCT GG-3'

5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC SAG GTC CAG CTG GTR CAG TCT GG-3'

5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG RTC ACC TTG AAG GAG TCT GG-3'

5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC SAG GTG CAG CTG GTG GAG TCT GG-3'

5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG GAG WCY GG-3'

5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTA CAG CAG TGG GG-3'

5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG STG CAG CTG CAG TCS GG-3'

5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAR GTG CAG CTG GTG CAG TCT GG-3'

5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG TCA GG-3'

FIG.2iv

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Oligonucleotide primers used for construction of the library

5'-TGA GGA GAC GGT GAC CAG GGT GCC-3'
5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

5'-ACC GCC TCC ACC AGT GCA CTT GAC ATC CAG WTG ACC CAG TCT CC-3'
5'-ACC GCC TCC ACC AGT GCA CTT GAT GTT GTG ATG ACT CAG TCT CC-3'
5'-ACC GCC TCC ACC AGT GCA CTT GAA ATT GTG WTG ACR CAG TCT CC-3'
5'-ACC GCC TCC ACC AGT GCA CTT GAT ATT GTG ATG ACC CAC ACT CC-3'
5'-ACC GCC TCC ACC AGT GCA CTT GAA ACG ACA CTC ACG CAG TCT CC-3'
5'-ACC GCC TCC ACC AGT GCA CTT GAA ATT GTG CTG ACT CAG TCT CC-3'

FIG.2v

Oligonucleotide primers used for construction of the library

5'-ACC GCC TCC ACC AGT GCA CAG TCT GTG CTG ACT CAG CCA CC-3'
5'-ACC GCC TCC ACC AGT GCA CAG TCT GTG YTG ACG CAG CCG CC-3'
5'-ACC GCC TCC ACC AGT GCA CAG TCT GTC GTG ACG CAG CCG CC-3'
5'-ACC GCC TCC ACC AGT GCA CAR TCT GCC CTG ACT CAG CCT-3'
5'-ACC GCC TCC ACC AGT GCA CTT TCC TAT GWG CTG ACT CAG CCA CC-3'
5'-ACC GCC TCC ACC AGT GCA CTT TCT TCT GAG CTG ACT CAG GAC CC-3'
5'-ACC GCC TCC ACC AGT GCA CAC GTT ATA CTG ACT CAA CCG CC-3'
5'-ACC GCC TCC ACC AGT GCA CAG GCT GTG CTG ACT CAG CCG TC-3'
5'-ACC GCC TCC ACC AGT GCA CTT AAT TTT ATG CTG ACT CAG CCC CA-3'
5'-ACC GCC TCC ACC AGT GCA CAG RCT GTG GTG ACY CAG GAG CC-3'
5'-ACC GCC TCC ACC AGT GCA CWG CCT GTG CTG ACT CAG CCM CC-3'

FIG.2vi